



D E C L A R A T I O N

In the matter of U.S. Patent
Application Ser. No. 10/070,387
in the name of Naoki MIDOH, et al.

I, Kyoko IMAMURA, of Kyowa Patent and Law Office, 2-3,
Marunouchi 3-Chome, Chiyoda-Ku, Tokyo-To, Japan, declare
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that I am thoroughly conversant with both the Japanese
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I further declare that all statements made herein of
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SPECIFICATION

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[TITLE OF INVENTION] CYCLIC DEPSIPEPTIDE SYNTHETASE GENE

[SCOPE OF CLAIMS]

[Claim 1]

A gene carrying the following DNA (a) or (b):

(a) DNA of a nucleotide sequence shown as SQ ID No. 1;

(b) a microorganism-derived DNA hybridizing with the DNA of the nucleotide sequence (a) under stringent conditions and encoding a protein with cyclic depsipeptide synthetase activity.

[Claim 2]

A protein described below in (a) or (b):

(a) a protein of an amino acid sequence shown as SQ ID No. 2; or

(b) a protein of an amino acid sequence prepared through deletion, substitution or addition of one or several amino acids in the amino acid sequence (a) and with cyclic depsipeptide synthetase activity.

[Claim 3]

A recombinant vector carrying a gene according to claim 1.

[Claim 4]

A microorganism harboring a recombinant vector according to claim 3 and expressing the gene.

[Claim 5]

A microorganism according to claim 4, wherein the microorganism is a fungus producing the substance PF1022.

[Claim 6]

A method for producing the substance PF1022 and a derivative thereof by using a microorganism according to claim 4 or 5.

[DETAILED DESCRIPTION OF THE INVENTION]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to the cyclic depsipeptide synthetase gene for producing the substance PF1022 as a cyclic depsipeptide with anthelmintic activity, a protein encoded by the gene, a recombinant vector using the gene, and a method for producing the substance PF1022 in a substance PF1022-producing microorganism integrated with the recombinant vector.

[0002]

[Prior Art]

The substance PF1022 [cyclo(D-lactyl-L-N-methyllleucyl-D-3-phenyllactyl-L-N-methyllleucyl-D-lactyl-L-N-methyllleucyl-D-3-phenyllactyl-L-N-methyllleucyl)] is the cyclic depsipeptide produced by a filamentous fungus of Agonomycetales, namely the strain PF1022 [Mycelia sterilia; the strain was deposited as FERM BP-2671 at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, Japan]. The substance PF1022 exerts an extremely high anthelmintic activity on nematodes parasitic on animals [Japanese Patent publication No. 35796/1991; Sasaki, T. et al., J. Antibiotics., 45, 692 (1992)]. Therefore, the substance is useful as an anthelmintic for animals and is additionally useful as a raw material for the synthesis of a more highly active derivative of the substance.

[0003]

The amounts of secondary metabolites produced by microorganisms and separated from natural origins are generally very slight. So as to industrially utilize such metabolites, therefore, the productivity of the secondary metabolites is necessarily increased. For the increase of the productivity, the examination of the culturing methods and the culture medium

components, the modification of the fermentation conditions such as addition of the precursors, and the mutation of the strains by ultraviolet irradiation or mutagen are carried out. In recent years, the productivity has been increased by utilizing gene recombinant technology in addition to the aforementioned methods.

[0004]

As the method, the enhancement of the expression of the genes of enzymes involved in the biosynthetic pathways, the enhancement of the expression of the genes involved in the control of the biosynthesis, and blockage of unnecessary biosynthetic pathways and the like are practically effected [Khetan, A. and Hu, W.-S. Manual of Industrial Microbiology and Biotechnology 2nd edition, p. 717, (1999)]. As a specific example, further, it has been known a method for improving the productivity, comprising allowing the hemoglobin gene of a bacterium to be expressed for the purpose of the improvement of the oxygen usability [Minas, W. et al., Biotechnol. Prog. 14, 561, (1998)].

[0005]

The most general means for the improvement of the productivity by gene recombinant technology is the enhancement of the expression of the genes of enzymes involved in biosynthetic pathway. For the application of the means, essentially, the transformation method of a subject microorganism is established; a promoter and a terminator applicable to the enhancement of the expression are to be present; and the biosynthetic pathway is to be elucidated and their genes are to be isolated. The transformation method of a fungus producing the substance PF1022, comprising integrating a foreign gene, has already been established (WO 97/00944); and also, a report concerning the promoter and terminator

applicable to the enhancement of the expression has been issued (Japanese Patent Application No. 252851/1999). However, no gene involved in the biosynthetic pathway has been isolated.
[0006]

The substance PF1022 is of a structure comprising L-N-methyllleucine, D-lactic acid, and D-phenyllactic acid, which are bonded together through ester bonds and amide bonds. It is considered that the substance is synthesized from 4 molecules of L-leucine, 2 molecules of D-lactic acid and 2 molecules of D-phenyllactic acid by a certain peptide synthetase in a fungus producing the substance. Peptide synthetase means an enzyme involved in the biosynthesis of microbial secondary metabolites, such as peptide, depsipeptide, lipopeptide and peptide lactone, from substrates of amino acids and hydroxy acid. The sequences of some peptide synthetases have already been elucidated [Marahiel, M. A. et al., Chem. Rev., 97, 2651, (1997)]. The reaction with such enzyme is totally different from the synthetic system of protein with ribosome using mRNA as template. Peptide synthetase has one domain for each substrate, where each substrate is activated with ATP for binding through phosphopantothenic acid in the domain; and the resulting bound substrates form amide bonds or ester bonds due to the catalytic actions in the regions between the individual domains.

[0007]

[Problem to be solved by the invention]

The cyclic depsipeptide synthetase gene capable of improving the productivity of the substance PF1022 is provided, by permitting excess expression thereof in a fungus producing the substance PF1022.

[0008]

[Means for Solving the Problem]

So as to overcome the problem, the inventors isolated the cyclic depsipeptide synthetase gene for the synthesis of the substance PF1022 from a fungus producing the substance PF1022, on the basis of the sequence of the conserved region of a known peptide synthetase. Furthermore, the inventors successfully improved the productivity of the substance PF1022 by preparing an expression vector modified with the promoter and terminator of the gene and integrating the expression vector in the fungus producing the substance PF1022. Thus, the invention has been achieved.

[0009]

More specifically, the invention relates to the following aspects.

1. A gene carrying the following DNA (a) or (b):
 - (a) DNA of a nucleotide sequence shown as SQ ID No. 1; or
 - (b) a microorganism-derived DNA hybridizing with the DNA of the nucleotide sequence (a) under stringent conditions and encoding a protein with cyclic depsipeptide synthetase activity.
2. A protein described below in (a) or (b):
 - (a) a protein of an amino acid sequence shown as SQ ID No. 2; or
 - (b) a protein of an amino acid sequence prepared by deletion, substitution or addition of one or several amino acids in the amino acid sequence (a) and with cyclic depsipeptide synthetase activity.
3. A recombinant vector carrying a gene in a first aspect of the invention.
4. A microorganism harboring a recombinant vector in a third aspect of the invention and expressing the gene.
5. A microorganism in a fourth aspect of the invention, wherein the microorganism is a fungus producing the substance PF1022.

6. A method for producing the substance PF1022 and a derivative thereof by using a microorganism in a fourth or fifth aspect of the invention.

[0010]

[Mode for Carrying out the Invention]

The cyclic depsipeptide synthetase gene of the invention can be isolated from a fungus producing the substance PF1022 for example by the following method.

[0011]

A library comprising the genomic DNA of a fungus producing the substance PF1022 is prepared by extracting the genomic DNA from the fungus producing the substance PF1022, cleaving the DNA with an appropriate restriction endonuclease, and subsequently ligating to a phage vector.

Based on the conserved region of the amino acid sequence of a known peptide synthetase and a partial amino acid sequence of the cyclic peptide synthetase purified from the fungus producing the substance PF1022, an appropriate primer is synthesized, which is used to effect polymerase chain reaction (PCR) with the genomic DNA derived from the fungus producing the substance PF1022 as template, to amplify a DNA fragment of the cyclic peptide synthetase gene. Using the DNA fragment as probe, the genome library is screened. In such manner, the whole region of the cyclic peptide synthetase gene can be isolated. After the determination of the nucleotide sequence of the DNA fragment, appropriate restriction cleavage sites are introduced upstream the translation initiation codon and downstream the translation termination codon by means such as PCR, to recover a gene fragment singly containing the cyclic peptide synthetase gene.

[0012]

The gene of the invention encompasses a nucleotide

sequence hybridizable with the thus determined nucleotide sequence under stringent conditions. Using routine methods (for example site-directed mutagenesis) in the field of genetic engineering, additionally, DNA fragments with modification of the gene, such as addition, insertion, deletion or substitution of the gene, can be encompassed within the scope of the invention. The stringent conditions herein referred to mean that the rinsing procedure of the membrane after hybridization is carried out in solutions at low salt concentrations and high temperature, for example, a condition such that rinsing is effected in 0.2 x SSC (1 x SSC: 15 mM citrate trisodium, 150 mM sodium chloride) - 0.1 % SDS solution at 60°C for 15 minutes.

[0013]

A promoter is conjugated upstream the cyclic peptide synthetase gene isolated by the method, while a terminator is conjugated downstream the cyclic peptide synthetase gene; additionally, selective marker genes such as nutrient auxotrophic complementary genes or/and genes with chemical resistance are conjugated thereto, to prepare a recombinant vector for gene expression.

[0014]

The selective markers for use in recombinant vectors for gene expression include for example nutrient auxotrophic complementary genes such as pyrG, argB, trpC, niaD, TRP1, LEU2 and URA3; and genes with chemical resistance against destomycin, benomil, oligomycin, hygromycin, G418, bleomycin, fleomycin, phosphinothricin, ampicillin, and kanamycin.

[0015]

The conjugation of the promoter and terminator to the inventive gene and the insertion thereof into a vector can be carried out by methods known per se. The promoter and terminator for use in accordance with the invention are not

specifically limited, and include for example, genes of glycolytic enzymes, such as 3-phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase and enolase; genes of amino acid synthesis, such as ornithine carbamoyltransferase and tryptophan synthase; genes of hydrolases, such as amylase, protease, lipase, cellulase and acetoamidase; genes of oxidoreductases, such as nitrate reductase, orotidine-5'-phosphate dehydrogenase, and alcohol dehydrogenase; and genes of bacteria producing the substance PF1022, which are highly expressed in the fungus producing the substance PF1022, such as Abp1.

[0016]

The transformation of a host with a recombinant vector prepared in such manner and the culturing of the resulting transformant enable prominent production of the substance PF1022. For a host with no synthesis of L-leucine, D-lactic acid or D-phenyllactic acid as a substrate for the cyclic depsipeptide synthetase of the invention, additionally, the host is cultured after addition of deficient substrates or derivatives thereof, whereby the substance PF1022 or derivatives thereof can be produced. The invention also encompasses the culturing of the transformant in a culture medium to collect the substance PF1022 or a derivative thereof from the resulting microorganisms.

[0017]

As the host for use, appropriate bacteria or fungal microorganisms usable as hosts for gene recombination can be used, with no specific limitation. Preferably, the host is Escherichia coli, a bacterium of the genus Bacillus, an actinomycetes, yeast and a filamentous fungus; more preferably, the host is a filamentous fungus producing the substance PF1022; and most preferably, the host is the strain PF1022 (Mycelia

sterilia, FERM BP-2671).

[0018]

The transformation of such host can be carried out by methods known per se. For example, the introduction of a recombinant vector for gene expression into a host can be carried out by routine methods, for example electroporation process, polyethylene glycol process, Agrobacterium process, lithium process, calcium chloride process and the like, with no specific limitation.

[0019]

The transformant can also be cultured by general methods, by appropriately selecting culture media and culturing conditions and the like. As the culture media, use can be made of routine components, such as carbon sources for example glucose, sucrose, thick malt syrup, dextrin, starch, glycerol, molasses, animal and vegetable oils and the like. As the nitrogen source, additionally, use can be made of soy bean powder, wheat germ, pharmamedia, corn steep liquor, cotton seed bran, meat extract, polypeptone, malt extract, yeast extract, ammonium sulfate, sodium nitrate, urea and the like. Additionally, it is effective to add inorganic salts capable of generating sodium, potassium, calcium (calcium carbonate and the like), magnesium, cobalt, chloride, phosphorus (dipotassium hydrogen phosphate), sulfuric acid (magnesium sulfate and the like) and other ions, if necessary. If necessary, furthermore, selective chemical agents including various vitamins such as thiamine (thiamine chloride salt and the like), amino acids such as glutamic acid (sodium glutamate and the like) and asparagine (DL-asparagine and the like), trace nutrients such as nucleotide and antibiotics can be added. Organic materials and inorganic materials supporting fungal growth and promoting the production of the substance PF1022 or

a derivative thereof can appropriately be added.

[0020]

The pH of the culture medium is about pH 6 to pH 8. As the culturing method, agitation culture, aerated agitation culture or submerged aerobic culture under aerobic conditions can be carried out. Particularly, submerged aerated culture is the most appropriate. The temperature appropriate for culturing is 15°C to 40°C. In many cases, the microorganism grows around 26°C to 37°C. The production of the substance PF1022 or a derivative thereof varies, depending on the culture medium and culture conditions or the host used, but the accumulation thereof generally reaches the peak in 2 days to 25 days by any of the culture methods. Just when the amount of the substance PF1022 or a derivative thereof reaches the peak, the culturing is terminated, to isolate and purify the objective substance from the culture.

[0021]

So as to recover the substance PF1022 or a derivative thereof from the culture, general separation means utilizing the characteristic properties, for example solvent extraction method, ion exchange resin method, adsorption or partition column chromatography method, gel filtration method, dialysis method, precipitation method and crystallization method, can be used singly or in appropriate combinations thereof to extract and purify the substance PF1022 or a derivative thereof. From the culture, for example, the substance PF1022 or a derivative thereof is extracted in acetone, methanol, butanol, ethyl acetate, butyl acetate and the like. So as to further purify the substance PF1022 or a derivative thereof, chromatography using Sephadex LH-20 (Pharmacia Co.) or Toyopearl HW-40 (Toso, Co.) is satisfactorily effected. By the methods described above or combinations thereof, the substance PF1022 or a

derivative thereof can be recovered in purity.

[0022]

[Examples]

The present invention will now be described in the following examples, but the invention is not limited to them.

[0023]

Example 1

Cloning of cyclic depsipeptide synthetase gene from a fungus producing the substance PF1022

1. Genomic DNA isolation and preparation of genome library
With UV irradiation or NTG treatment, mutation was induced into the fungus (Mycelia sterilia; FERM BP-2671) producing the substance PF1022, to prepare a fungal strain 432-26 producing the substance PF1022 and having an improved productivity of PF1022, from which the genome DNA was extracted. The fungal strain 432-26 producing the substance PF1022 was cultured in 50-ml of a seed culture medium [1 % yeast extract, 1 % malt extract, 2 % polypeptone, 2.5 % glucose, 0.1 % dipotassium hydrogen phosphate, 0.05 % magnesium sulfate (pH 7.0)] at 26°C for 2 days, to recover the fungi by centrifugation. The resulting fungi were frozen in liquid nitrogen and ground in a mortar with a pestle. From the ground fungi, the genome DNA was isolated by ISOPLANT (Nippon Gene, Co.) according to the attached protocol. The isolated genome DNA was partially digested with Sau3A I, to recover DNA fragments of 15 kb to 20 kb by agarose gel electrophoresis, which were then treated with alkali phosphatase to dephosphorylate the termini of the DNA fragments. The DNA fragments were inserted in a phage vector Lambda DASH II (STRATAGENE, CO.). The recombinant phage vector recovered in such manner was subjected to in vitro packaging with Gigapack III Gold Packaging Extract (STRATAGENE CO.) according to the attached protocol. Subsequently, the

recombinant phage grew on the host Escherichia coli strain XL1-Blue MRA (P2) for plaque formation on a plate.

[0024]

2. Isolation of partial DNA fragment of cyclic depsipeptide synthetase gene

A known peptide synthetase was subjected to multiple alignment, so that WTSMYDG and VVQYFPT were detected as excellently conserved regions. Based on these sequences, primers 5'-TGGACIWSNATGTAYGAYGG-3' (SQ ID NO. 3) and 5'-GTIGGRAARTAYTGIACNAC-3' (SQ ID NO. 4) were synthesized. Using these primers, the genome DNA isolated from the fungus producing the substance PF1022 was used as template for PCR. Using 50 ng of the genome DNA as template, 1 .25 units ExTaq DNA polymerase (TaKaRa Brewery, Co.), the attached buffer and dNTP mixture, and 10 μ M primer in 50 μ l of a reaction solution, PCR was conducted under the following conditions: 94°C for 3 minutes [94°C for one minute, 65°C (with 0.5°C decrement per one cycle) for one minute, 72°C for one minute] x 30 times and 72°C for 3 minutes. Through the reaction, a DNA fragment of about 350 bp was amplified; and the resulting DNA fragment was inserted in pCR2.1 plasmid vector, by using Original TA Cloning Kit (Invitrogen Co.) according to the attached protocol.

[0025]

The nucleotide sequence of the thus cloned DNA fragment was determined, by using DNA sequencing Kit dRhodamine Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Co.) and ABI PRISM 310 Genetic Analyzer (Perkin Elmer Co.) according to the attached protocol. Consequently, the nucleotide sequence of the isolated DNA fragment was homologous to the peptide synthetase gene, which apparently indicates that the DNA fragment was a part of the objective cyclic depsipeptide synthetase gene.

[0026]

3. Cloning of the whole region of the cyclic depsipeptide synthetase gene

The probe for use in the screening of the genome library was prepared, by PCR to allow the DNA fragment to incorporate fluorescein-labeled dUTP. By using pCR2.1 plasmid vector inserted with 100 ng of the DNA fragment of the cyclic depsipeptide synthetase gene as template, 1.25 units ExTaq DNA polymerase (TaKaRa Brewery, Co.) and the attached buffer, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dGTP, 0.02 mM dTTP, 0.18 mM fluorescein-labeled dUTP (FluoroGreen; Amersham Co.) and 10 μ M primers (SQ ID Nos. 3 and 4) in 50 μ l of a reaction solution, PCR was carried out under the following conditions: 94°C for 2 minutes, (94°C for 30 seconds, 55°C for one minute and 72°C for one minute) x 25 times, 72°C for 3 minutes. Through the reaction, a fluorescein-labeled probe of about 350 bp was prepared.

[0027]

On the plate with formed plaques as prepared in Example 1.1, Hybond-N+ membrane (Amersham Co.) was mounted, to deposit the plaques. The membrane was treated with an alkali, to denature the recombinant phage DNA on the membrane into a single strand and thereby adsorb the DNA on the membrane. The phage DNA-adsorbed membrane was placed in a buffer prepared by using Hybridization Buffer Tablets (Amersham Co.), for incubation at 60°C for one hour. The fluorescein-labeled probe was denatured and added to the resulting incubation mixture, for overnight hybridization at 60°C. Thereafter, the membrane was rinsed in 1 x SSC (SSC: 15 mM citrate trisodium, 150 mM sodium chloride) -0.1 % SDS solution at 60°C for 15 minutes and further rinsed in 0.2 x SSC-0.1 % SDS solution at 60°C for 15 minutes. The

fluorescein-bound plaque was visualized by using DIG-wash and block buffer set (Boehringer Mannheim, Co.), alkali phosphatase-labeled anti-fluorescein antibody (Anti-fluorescein-AP, Fab fragment, Boehringer Mannheim Co.), a chromogenic substrate nitroblue tetrazolium chloride (Boehringer Mannheim Co.) and X-phosphate (Boehringer Mannheim Co.) according to the attached protocol. In such manner, a positive clone carrying the 5' upstream region and 3' downstream region of a region homologous to the probe was screened.

[0028]

4. Determination of the nucleotide sequence

The DNA fragment in the positive clone thus isolated was amplified by PCR using primers 5'-GCGGAATTAACCCTCACTAAAGGGAACGAA-3' (SQ ID No. 5) and 5'-GCGTAATACGACTCACTATAGGGCGAAGAA-3' (SQ ID No. 6) as the phage vector sequences. Using 100 ng of the positive clone DNA as template, 2.5 units LA Taq DNA polymerase (TaKaRa Brewery, Co.), the attached buffer and dNTP mixture, 2.5 mM magnesium chloride, and 0.2 μ M primer in a reaction solution of 50 μ l, PCR was effected under the following conditions: 94°C for one minute, (98°C for 10 seconds and 68°C for 15 minutes) x 25 times, 72°C for 15 minutes. The resulting PCR product was purified and treated with a nebulizer, to be decomposed randomly into fragments of 0.5 kb to 2.0 kb. The termini of the resulting fragments were blunt ended with T4 DNA polymerase and phosphorylated with T4 polynucleotide kinase, to be then inserted at the EcoRV site of pT7Blue (Novagen Co.) for insertion in Escherichia coli strain JM109. 168 colonies thus prepared were directly subjected to PCR using M13 Primer M4 (TaKaRa Brewery, Co.) and M13 primer RV (TaKaRa Brewery, Co.) and were then purified, which were then sequenced by using M13 primer M4 (TaKaRa Brewery, Co.). Using 1.25 units ExTaq DNA polymerase (TaKaRa Brewery,

Co.), the attached buffer and dNTP mixture, and 0.5 μ M primer, PCR was effected in 50 μ l of a reaction solution under the following conditions: 94°C for 4 minutes, (94°C for 30 seconds, 55°C for 30 seconds and 72°C for 2 minutes) x 30 times, 72°C for 3 minutes. Additionally, sequencing was effected, by using DNA Sequencing Kit dRhodamine Terminator Cycle Sequencing Ready Reaction (Perkin Elmer Co.) and ABI PRISM 310 Genetic Analyzer (Perkin Elmer Co.) according to the attached protocol.

[0029]

Based on the results, an insufficiently analyzed region was amplified by PCR using a primer newly designed on the basis of the analyzed nucleotide sequence and was then purified. Using the primer used for PCR, the region was sequenced, whereby the 15606-bp nucleotide sequence of the DNA fragment in the positive clone was determined.

[0030]

The analysis of the sequence apparently indicates that an open reading frame (ORF) of 9633 bp was present and a protein speculated from the sequence comprised 3210 amino acid residues of 353 kDa and that the protein was homologous to the peptide synthetase. Additionally, the protein with the highest homology was enniatin synthetase (S39842) with 56 % homology. The nucleotide sequence and amino acid sequence of the ORF of the cyclic depsipeptide synthetase gene thus isolated in accordance with the invention are shown as SQ ID Nos. 1 and 2, respectively in the sequence listing.

[0031]

Example 2

Improvement of PF1022 productivity due to the excess expression of cyclic depsipeptide synthetase gene

1. Construction of recombinant vector for gene expression (Figure 1)

From the positive clone recovered in Example 1. 3, plasmid pPF7 was prepared by cleaving the inserted DNA fragment with NotI and inserting the DNA fragment into the NotI site of the pBluescriptII KS+ (STRATAGENE CO.). pPF7 was cleaved with BanIII and SmaI and electrophoresed on agarose gel, to recover a DNA fragment of about 8250 bp from the agarose gel. The fragment was inserted in pBluescriptII KS+, to prepare plasmid pPF7-1.

[0032]

Using pPF7 as template, 5'-AGCATCGGATCCTAACAATGGGCGTTGAGCAGCAAGCCCTA-3' (SQ ID No. 7; designed for the initiation of the translation at the 9th Met from the N terminus of the ORF) or 5'-AGCATCGGATCCTAACAATGTCAAACATGGCACCCTCCCTA-3' (SQ ID No. 13; designed for the initiation of the translation at the first Met from the N terminus of the ORF), and 5'-TTTGCTTCGTACTCGGGTCCT-3' (SQ ID No. 8) as primers for the amplification of a region of about 440 bp (using SQ ID Nos. 7 and 8) or a region of about 470 bp (using SQ ID Nos. 13 and 8) around the N terminus to the BanIII site, and 5'-GCATCGCGATACTAGAGAAG-3' (SQ ID No. 9) and 5'-AGCATCGAATTCGGATCCCTAAACCAACGCCAAAGCCCGAAT-3' (SQ ID No. 10) as primers for the amplification of a region of about 920 bp from the SmaI site to the C terminus, PCR was effected. Then, the primers were designed so as to insert the BamHI site at the 5' and 3' sites of the inventive cyclic depsipeptide synthetase gene. Using 150 ng of the plasmid DNA as template, 2.5 units KOD DNA polymerase (Toyo Boseki), the attached buffer and dNTP mixture, 1 mM magnesium chloride, and 0.5 μ M primer in 50 μ l of a reaction solution, PCR was effected under the following conditions : 98°C for 30 seconds, (98°C for 15 seconds, 65°C for 2 seconds, 74°C for 30 seconds) x 10 times, 74°C for one minute.

The PCR reaction solutions recovered by using the individual primers were precipitated with ethanol, to recover PCR products. Concerning the N terminal region, the N terminal region was cleaved with BamHI and BanIII; concerning the C terminal region, the C terminal region was cleaved with SmaI and BamHI. Thereafter, the resulting fragments were electrophoresed on agarose gel, to recover DNA fragments from the agarose gel. [0033]

The PCR fragment of the C terminal region was inserted in the SmaI and BamHI sites of pPF7-1, to prepare plasmid pPF7-2. The plasmid was cleaved with BanIII and BamHI and electrophoresed on agarose gel, to recover a DNA fragment of about 9170 bp from the agarose gel. The DNA fragment and the N terminal region prepared by using SQ ID Nos. 7 and 8 were simultaneously inserted in the BamHI site of pBluescript II KS+, to reconstruct the cyclic depsipeptide synthetase gene of the invention and prepare plasmid pPFsyn (initiating the translation at the 9th Met from the N terminus of the ORF). [0034]

Alternatively, an about 9170-bp DNA fragment cleaved from pPF7-2 and the N-terminal region prepared by using SQ ID Nos. 7 and 13 were simultaneously inserted in the BamHI site of pHSG299 (TaKaRa Brewery Co.), to reconstruct the cyclic depsipeptide synthetase gene of the invention and prepare plasmid pPFsynN (initiating the translation at the first Met from the N terminus of the ORF). In such manner, the cyclic depsipeptide synthetase gene with the BamHI sites on both the termini was prepared.

[0035]

Herein, Escherichia coli transformed with the plasmid pPFsyn (Escherichia coli DH5 α) was deposited with Accession No. FERM P-17541 at National Institute of Bioscience and

Human-Technology, Agency of Industrial Science and Technology, supra.

[0036]

Additionally, Escherichia coli transformed with the plasmid pPFsynN (Escherichia coli DH5 α) was deposited with Accession No. FERM P-17542 at National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, supra.

[0037]

pPFsyn or pPFsynN was cleaved with BamHI. Subsequently, the cyclic depsipeptide synthetase gene region was recovered from the individual gels. The gene region was inserted at the BamHI site of pABPd with the promoter and terminator of Abp1 gene described in Japanese Patent Application No. 252851/1999, to prepare an expression vector pABP/PFsyn (initiating the translation at the 9th Met of the N terminus of ORF) and an expression vector pABP/PFsynN (initiating the translation at the first Met of the N terminus of ORF) as the expression vectors for expressing the cyclic depsipeptide synthetase gene of the invention.

[0038]

2. Introduction and expression of cyclic depsipeptide synthetase gene in fungus producing the substance PF1022
The expression vectors were introduced in a fungus producing the substance PF1022 (Mycelia sterilia; FERM BP-2671) according to the method of Example 1 described in WO 97/00944, to screen strains with high resistance against hygromycin B. The introduction of the objective gene in these strains was confirmed by PCR using a primer 5'-TGATATGCTGGAGCTTCCCT-3' (SQ ID No. 11) prepared from the sequence of Abp1 promoter and a primer 5'-GCACAACCTCTTCCAGGCT-3' (SQ ID No. 12) prepared from the sequence of the cyclic depsipeptide synthetase gene. In

such manner, gene-introduced strains with high resistance against hygromycin B and with the inventive cyclic depsipeptide synthetase gene introduced therein were screened.

[0039]

The gene-introduced strains and the parent strain (Mycelia sterilia; FERM BP-2671) were separately cultured in 50 ml of a seed culture medium at 26°C for 2 days; 1 ml of each of the cultures was inoculated separately in a generation culture medium [6 % thick malt syrup, 2.6 % starch, 2 % wheat germ, 1 % pharmedia, 0.2 % magnesium sulfate 7 hydrates, 0.2 % calcium carbonate, 0.3 % sodium chloride (pH 7.5)] and cultured therein at 26°C for 4 days. The resulting culture was centrifuged at 4500 rpm for 5 minutes to harvest the fungus; the resulting individual fungus species were rinsed in 0.3 M potassium chloride. The individual fungal species were frozen in liquid nitrogen and freeze-dried.

[0040]

The following extraction procedure was carried out on ice or in a low-temperature chamber at 4°C. Into a 2-ml tube containing 10 mg of the freeze-dried fungal species and 1.0 ml of glass beads (0.5-mm diameter) was added 1.0 ml of an extraction buffer [50 mM Tris-HCl (pH 8.0), 0.3 M potassium chloride, 60 % glycerol, 10 mM ethylenediaminetetraacetate disodium, 5 mM dithiothreitol, 10 µM leupeptin, 1 mM phenylmethanesulfonic acid, 60 µg/ml chimostatin]. The microtube was set in a Mini-Bead-Beater-8 (Biospec, Co.), which was then driven at the maximum speed for 3 minutes for extraction. The resulting mixture was centrifuged at 15000 rpm for 5 minutes; 100 µl of the supernatant was charged in and mixed with 100 µl of 10 % trichloroacetic acid solution. After the solution was left to stand for 15 minutes, the solution was centrifuged at 15000 rpm for 10 minutes. The resulting

precipitate was dissolved in 15 µl of an alkali solution (2 % sodium carbonate, 0.4 % sodium hydroxide), to which was added 60 µl of a sample buffer [125 mM Tris-HCl (pH 6.8), 20 % glycerol, 4 % sodium dodecylsulfate, 10 % 2-mercaptoethanol, 50 mg/1 bromophenol blue]. This was heated in boiling water for 5 minutes, and was then electrophoresed [Sodium Dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)] on 4 % to 20 % polyacrylamide gel with an electrophoresis system (Tefco Co.). The polyacrylamide gel after electrophoresis was stained by using Quick-CBB (Wako Pure Chemical Co.) according to the attached protocol. The electrophoresis result of the proteins extracted from the parent strain and the gene-introduced strain with pABP/PFsyn is shown in Figure 2.

[0041]

As described above, the expression level of the cyclic depsipeptide synthetase in the gene-introduced strains was distinctively higher than the level in the parent strain.

[0042]

3. Extraction and assay of the substance PF1022

The gene-introduced strains and the parent strain were separately cultured in 50 ml of a seed culture medium at 26°C for 2 days; and 1 ml of each of 50 ml of the cultures was inoculated separately in a generation culture medium and cultured therein at 26°C for 6 days. 10 ml each of the cultures was placed and centrifuged at 3000 rpm for 10 minutes; and the resulting strains were harvested separately. 10 ml of methanol was added to the individual strains, which were vigorously shaken and left to stand for 30 minutes. Thereafter, those were again shaken and centrifuged at 3000 rpm for 10 minutes; the substance PF1022 extracted from the individual strains in the supernatant was assayed by liquid chromatography. As the column, LiChrospher 100 RP-18 (e) (Kanto Chemical CO.) was used;

the column temperature was 40°C; the mobile phase was 80 % acetonitrile at a flow rate of 1.0 ml/min; the substance PF1022 was detected on the basis of the absorbance at 210 nm. The retention time of the substance PF1022 under the conditions was 5 minutes to 6 minutes. The experiments were carried out in duplicate. The average values of the assay results of the substance PF1022 extracted from the parent strain and the gene-introduced strain with the pABP/PFsyn are shown in Table 1.

[0043]

[Table 1]

	Substance PF1022 (μg/ml)
Parent strain	88
Gene-introduced strain	222

[0044]

The substance PF1022 productivity of the gene-introduced strain was about 2.5-fold that of the parent strain. It is apparently shown that the excess expression of the inventive cyclic depsipeptide synthetase elevates the productivity of the substance PF1022.

[0045]

[ADVANTAGEOUS EFFECTS OF THE INVENTION]

The introduction of the inventive cyclic depsipeptide synthetase gene in the fungus producing the substance PF1022 can improve the productivity of the substance PF1022.

[0046]

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Tyr Ala Asp Leu Asp Arg Gln Ser Asp Gln Leu Ala Thr Trp Leu Gly

1585

1590

1595

cgg cgc aat atg acg gct gag acg ctg gtc ggg gtg tta gca ccg cgg 4848

Arg Arg Asn Met Thr Ala Glu Thr Leu Val Gly Val Leu Ala Pro Arg

1600

1605

1610

tca tgt caa aca gtt gtt gcc att tta ggt atc ctg aaa gcg aat ctc 4896

Ser Cys Gln Thr Val Val Ala Ile Leu Gly Ile Leu Lys Ala Asn Leu

1615

1620

1625

gca tat ctc ccg ctt gat gtg aat tgt cct acc gcc cgc ctg caa aca 4944

Ala Tyr Leu Pro Leu Asp Val Asn Cys Pro Thr Ala Arg Leu Gln Thr

1630

1635

1640

atc cta tct aca ttg aat cgg cac aag ttg gtc cta ctc ggc tct aac 4992

Ile Leu Ser Thr Leu Asn Arg His Lys Leu Val Leu Leu Gly Ser Asn

1645

1650

1655

1660

gca act act ccg gat gtc cag ata cct gat gta gag ctg gta cga atc 5040

Ala Thr Thr Pro Asp Val Gln Ile Pro Asp Val Glu Leu Val Arg Ile

1665

1670

1675

agc gat atc tta gat cgc ccc atc aat ggc cag gca aag cta aat ggt 5088

Ser Asp Ile Leu Asp Arg Pro Ile Asn Gly Gln Ala Lys Leu Asn Gly

1680

1685

1690

cat aca aaa tcg aat ggc tac tca aag cca aac ggc tat acg cat ctg 5136

His Thr Lys Ser Asn Gly Tyr Ser Lys Pro Asn Gly Tyr Thr His Leu

1695

1700

1705

aaa ggc tat tca aac cta aac ggt tat tca aaa caa aat ggt tat gca 5184

Lys Gly Tyr Ser Asn Leu Asn Gly Tyr Ser Lys Gln Asn Gly Tyr Ala

1710

1715

1720

caa ctc aac ggc cat aga gag cgt aac aat tat tta gat cta aat ggg 5232

Gln Leu Asn Gly His Arg Glu Arg Asn Asn Tyr Leu Asp Leu Asn Gly

1725

1730

1735

1740

cac tca ctg cta aat ggg aat tca gac atc acc aca tca ggg ccc tca 5280

His Ser Leu Leu Asn Gly Asn Ser Asp Ile Thr Thr Ser Gly Pro Ser

1745

1750

1755

gca aca agc ctt gcc tac gtg atc ttc aca tcc ggc tca acc gga aag 5328

Ala Thr Ser Leu Ala Tyr Val Ile Phe Thr Ser Gly Ser Thr Gly Lys

1760

1765

1770

ccc aaa gga gtc atg gtc gaa cac cgc agc atc atc cga ctt gca aag 5376

Pro Lys Gly Val Met Val Glu His Arg Ser Ile Ile Arg Leu Ala Lys

1775

1780

1785

aag aac aga atc ata tcc agg ttc cca tct gta gcc aag gta gct cac 5424

Lys Asn Arg Ile Ile Ser Arg Phe Pro Ser Val Ala Lys Val Ala His

1790

1795

1800

ctc tca aac atc gcc ttt gac gcc gcc act tgg gaa atg ttc gca gcc 5472

Leu Ser Asn Ile Ala Phe Asp Ala Ala Thr Trp Glu Met Phe Ala Ala

1805

1810

1815

1820

ctt cta aac ggc gga acg ctg gtc tgt atc gac tat atg acc acc ctg 5520

Leu Leu Asn Gly Gly Thr Leu Val Cys Ile Asp Tyr Met Thr Thr Leu

1825

1830

1835

gac agc aaa acg ctc gag gcc gcg ttt gca cga gaa caa atc aac gcc 5568

Asp Ser Lys Thr Leu Glu Ala Ala Phe Ala Arg Glu Gln Ile Asn Ala

1840

1845

1850

gcg tta ctc acg ccc gct ttg ttg aag cag tgc cta gcc aac att ccc 5616

Ala Leu Leu Thr Pro Ala Leu Leu Lys Gln Cys Leu Ala Asn Ile Pro

1855

1860

1865

act acc cta ggc agg ctg agt gca ctc gtt att gga ggt gat agg ctt 5664

Thr Thr Leu Gly Arg Leu Ser Ala Leu Val Ile Gly Gly Asp Arg Leu

1870

1875

1880

gac ggc caa gac gcg atc gca gca cat gcg ctt gtc ggt gct ggc gtg 5712

Asp Gly Gln Asp Ala Ile Ala Ala His Ala Leu Val Gly Ala Gly Val

1885

1890

1895

1900

tat aat gcg tat ggc ccg acc gaa aac gga gtg atc agt acg att tat 5760

Tyr Asn Ala Tyr Gly Pro Thr Glu Asn Gly Val Ile Ser Thr Ile Tyr

1905

1910

1915

aat atc act aaa aac gac tcg ttc atc aac gga gtc ccc atc ggc tgt 5808

Asn Ile Thr Lys Asn Asp Ser Phe Ile Asn Gly Val Pro Ile Gly Cys

1920

1925

1930

gca atc agc aat tcc ggc gcc tac atc aca gac cca gac cag cag ctc 5856

Ala Ile Ser Asn Ser Gly Ala Tyr Ile Thr Asp Pro Asp Gln Gln Leu

1935

1940

1945

gta cct cct ggc gtc atg ggt gaa ctc gtc gtt acc ggt gac ggg ctc 5904

Val Pro Pro Gly Val Met Gly Glu Leu Val Val Thr Gly Asp Gly Leu

1950

1955

1960

gcg cgg ggg tat aca gac cca gca cta gac gcg ggc cgc ttc gtc cag 5952

Ala Arg Gly Tyr Thr Asp Pro Ala Leu Asp Ala Gly Arg Phe Val Gln

1965

1970

1975

1980

atc atg atc aat gac aag gcc gtg agg gcg tac cga acg ggt gac cgg 6000

Ile Met Ile Asn Asp Lys Ala Val Arg Ala Tyr Arg Thr Gly Asp Arg

1985

1990

1995

gca cga tat cgc gta gga gac ggt cag atc gag ttc ttc gga cgc atg 6048

Ala Arg Tyr Arg Val Gly Asp Gly Gln Ile Glu Phe Phe Gly Arg Met

2000

2005

2010

gat cag caa gtc aag atc cga ggt cac cgc att gaa cca gcc gaa gtg 6096

Asp Gln Gln Val Lys Ile Arg Gly His Arg Ile Glu Pro Ala Glu Val

2015

2020

2025

gag cgt gct att ctc gac caa gac tcg gcc cgc gac gcc gtc gtt gtc 6144

Glu Arg Ala Ile Leu Asp Gln Asp Ser Ala Arg Asp Ala Val Val Val

2030

2035

2040

atc cgg cac caa gaa ggt gaa gaa ccg gag atg gtt ggt ttc gtc gcg 6192

Ile Arg His Gln Glu Gly Glu Glu Pro Glu Met Val Gly Phe Val Ala

2045

2050

2055

2060

acc cac ggc gat cac tct gcc gaa caa gag gaa gca gac gac cag gtt 6240

Thr His Gly Asp His Ser Ala Glu Gln Glu Glu Ala Asp Asp Gln Val

2065

2070

2075

gaa ggt tgg aaa gac ttc ttc gag agc aat aca tat gcc gac atg gat 6288

Glu Gly Trp Lys Asp Phe Phe Glu Ser Asn Thr Tyr Ala Asp Met Asp

2080

2085

2090

acc atc ggc cag tct gct ata ggc aac gac ttt acg ggc tgg acg tcc 6336

Thr Ile Gly Gln Ser Ala Ile Gly Asn Asp Phe Thr Gly Trp Thr Ser

2095

2100

2105

atg tac gac ggg agc gag atc aac aag gcc gag atg cag gag tgg ctc 6384

Met Tyr Asp Gly Ser Glu Ile Asn Lys Ala Glu Met Gln Glu Trp Leu

2110

2115

2120

gac gac acc atg cgc aca ctc ctc gat ggc caa gcg ccg ggt cac gta 6432

Asp Asp Thr Met Arg Thr Leu Leu Asp Gly Gln Ala Pro Gly His Val

2125

2130

2135

2140

ctc gaa ata ggc aca ggc agt ggc atg gta ttg ttt aac tta ggg gcc 6480

Leu Glu Ile Gly Thr Gly Ser Gly Met Val Leu Phe Asn Leu Gly Ala

2145

2150

2155

ggg cta caa agc tac gta ggt ctt gaa cca tct aga tct gca gcc acg 6528

Gly Leu Gln Ser Tyr Val Gly Leu Glu Pro Ser Arg Ser Ala Ala Thr

2160

2165

2170

ttt gtt acc aaa gcg atc aat tcc acc cca gct ctt gca gga aag gcc 6576

Phe Val Thr Lys Ala Ile Asn Ser Thr Pro Ala Leu Ala Gly Lys Ala

2175

2180

2185

gaa gtg cac gtc ggc aca gcg aca gac ata aac cga ctt cgt gga ctt 6624

Glu Val His Val Gly Thr Ala Thr Asp Ile Asn Arg Leu Arg Gly Leu

2190

2195

2200

cgt ccc gat cta gtt gtg ctc aac tcg gta gtc cag tat ttc ccc acg 6672

Arg Pro Asp Leu Val Val Leu Asn Ser Val Val Gln Tyr Phe Pro Thr

2205

2210

2215

2220

ccc gag tac cta cta gag gtc gtg gag agt ctc gtc cgg att ccg ggc 6720

Pro Glu Tyr Leu Leu Glu Val Val Glu Ser Leu Val Arg Ile Pro Gly

2225

2230

2235

gtc aag cgc gtg gtc ttc ggc gac ata cga tct cac gcc acg aac aga 6768

Val Lys Arg Val Val Phe Gly Asp Ile Arg Ser His Ala Thr Asn Arg

2240

2245

2250

cat ttt ctt gct gcc agg gcg ctg cat tcg ctg ggc tcc aag gcg acc 6816

His Phe Leu Ala Ala Arg Ala Leu His Ser Leu Gly Ser Lys Ala Thr

2255

2260

2265

aaa gat gct ata cgt caa aag atg acg gag atg gaa gag cgc gag gaa 6864

Lys Asp Ala Ile Arg Gln Lys Met Thr Glu Met Glu Glu Arg Glu Glu

2270

2275

2280

gag ctg ctc gtc gac ccg gcc ttc ttc acg gcg ctg ctg cag ggc cag 6912

Glu Leu Leu Val Asp Pro Ala Phe Phe Thr Ala Leu Leu Gln Gly Gln

2285

2290

2295

2300

ctt gcc gat cga atc aag cac gtc gag atc ctc ccg aag aac atg cgc 6960

Leu Ala Asp Arg Ile Lys His Val Glu Ile Leu Pro Lys Asn Met Arg

2305

2310

2315

gcc acg aac gag ctg agc gcg tac cgg tat aca gcc gtc att cac gta 7008

Ala Thr Asn Glu Leu Ser Ala Tyr Arg Tyr Thr Ala Val Ile His Val

2320

2325

2330

cgc ggc cca gag gaa cag tcg cgg ccc gtg tat ccg atc caa gtg aac 7056

Arg Gly Pro Glu Glu Gln Ser Arg Pro Val Tyr Pro Ile Gln Val Asn

2335

2340

2345

gac tgg atc gac ttt cag gcc tca cgc att gac cgc cgc gcc ctt ctc 7104

Asp Trp Ile Asp Phe Gln Ala Ser Arg Ile Asp Arg Arg Ala Leu Leu

2350

2355

2360

cga ctc cta cag cgc tcg gca gac gcc gcg acc gtc gcc gtc agc aac 7152

Arg Leu Leu Gln Arg Ser Ala Asp Ala Ala Thr Val Ala Val Ser Asn

2365

2370

2375

2380

atc ccc tac agc aag acg att gta gaa cgc cat gtc gtc gag tcc ctt 7200

Ile Pro Tyr Ser Lys Thr Ile Val Glu Arg His Val Val Glu Ser Leu

2385

2390

2395

gac aat aac aac agg gag aat acg cat aga gca cca gac ggc gcg gct 7248

Asp Asn Asn Asn Arg Glu Asn Thr His Arg Ala Pro Asp Gly Ala Ala

2400

2405

2410

tgg atc tcg gcc gtc cgc tcc aag gcc gag cgc tgc acg tcc ctc tcc 7296

Trp Ile Ser Ala Val Arg Ser Lys Ala Glu Arg Cys Thr Ser Leu Ser

2415

2420

2425

gtg acc gat ctt gtg cag ctc ggg gaa gaa gcc ggc ttt cgc gta gaa 7344

Val Thr Asp Leu Val Gln Leu Gly Glu Glu Ala Gly Phe Arg Val Glu

2430

2435

2440

gtc agc gca gcg cgg cag tgg tct caa agc ggc gcg ctc gat gcc gtc 7392

Val Ser Ala Ala Arg Gln Trp Ser Gln Ser Gly Ala Leu Asp Ala Val

2445

2450

2455

2460

ttt cac cgc tat aat ttg ccc act caa agc aat agt cgc gtt ctg att 7440

Phe His Arg Tyr Asn Leu Pro Thr Gln Ser Asn Ser Arg Val Leu Ile

2465

2470

2475

cag ttc cct acc gaa gat ggc cag acg cga aga tcc gcc act ctg aca 7488

Gln Phe Pro Thr Glu Asp Gly Gln Thr Arg Arg Ser Ala Thr Leu Thr

2480

2485

2490

aac cga cca cta cag cgt ctg cag agc cgt cgg ttc gca tca cag atc 7536

Asn Arg Pro Leu Gln Arg Leu Gln Ser Arg Arg Phe Ala Ser Gln Ile

2495

2500

2505

cgc gaa cag ctg aag gcc gtg ctc ccg tca tac atg atc ccc tcc cgc 7584

Arg Glu Gln Leu Lys Ala Val Leu Pro Ser Tyr Met Ile Pro Ser Arg

2510

2515

2520

atc gtg gtc ata gac cag atg cct ctc aat gcc aat ggc aag gtc gac 7632

Ile Val Val Ile Asp Gln Met Pro Leu Asn Ala Asn Gly Lys Val Asp

2525

2530

2535

2540

cgg aaa gaa ctt acc aga agg gcc caa atc gcg ccg aaa tct cag gcg 7680

Arg Lys Glu Leu Thr Arg Arg Ala Gln Ile Ala Pro Lys Ser Gln Ala

2545

2550

2555

gct ccc gcc aaa ccc gtc aaa cag gtc gat ccg ttc gtc aac ctg gaa 7728

Ala Pro Ala Lys Pro Val Lys Gln Val Asp Pro Phe Val Asn Leu Glu

2560

2565

2570

gcc att tta tgt gag gag ttc gcg gag gtg ctg ggc atg gaa gtc ggc 7776

Ala Ile Leu Cys Glu Glu Phe Ala Glu Val Leu Gly Met Glu Val Gly

2575

2580

2585

gtg aac gac cac ttc ttc caa cta ggc gga cac tct ctc ttg gcc acg 7824

Val Asn Asp His Phe Phe Gln Leu Gly Gly His Ser Leu Leu Ala Thr

2590

2595

2600

aag ctc gtc gcg cgt ctc agt cgt cgg cta aac ggt cgt gtg tct gtg 7872

Lys Leu Val Ala Arg Leu Ser Arg Arg Leu Asn Gly Arg Val Ser Val

2605

2610

2615

2620

agg gat gtg ttc gac cag cct gtg att tcc gac ctc gca gtc acc ctc 7920

Arg Asp Val Phe Asp Gln Pro Val Ile Ser Asp Leu Ala Val Thr Leu

2625

2630

2635

cgc cag gga ctg acc ttg gaa aac gcc att ccc gca acg ccg gac agc 7968

Arg Gln Gly Leu Thr Leu Glu Asn Ala Ile Pro Ala Thr Pro Asp Ser

2640

2645

2650

ggg tat tgg gag cag aca atg tcc gca ccg aca acc ccg agc gac gac 8016

Gly Tyr Trp Glu Gln Thr Met Ser Ala Pro Thr Thr Pro Ser Asp Asp

2655

2660

2665

atg gag gcc gtg cta tgc aag gag ttt gcg gat gtg ctt ggc gtc gaa 8064

Met Glu Ala Val Leu Cys Lys Glu Phe Ala Asp Val Leu Gly Val Glu

2670

2675

2680

gtc agc gcc acc gac agc ttc ttc gat ctc ggt ggg cat tcc ctc atg 8112

Val Ser Ala Thr Asp Ser Phe Phe Asp Leu Gly Gly His Ser Leu Met

2685

2690

2695

2700

gct acg aag ctc gct gcg cgt att agc cgt cgg cta gat gta ccg gtg 8160

Ala Thr Lys Leu Ala Ala Arg Ile Ser Arg Arg Leu Asp Val Pro Val

2705

2710

2715

tca atc aaa gac ata ttc gat cac tca gtt cct cta aac ctt gcg agg 8208

Ser Ile Lys Asp Ile Phe Asp His Ser Val Pro Leu Asn Leu Ala Arg

2720

2725

2730

aag att cgg ctc act caa gca aaa ggc cac gaa gcg acc aat gga gta 8256

Lys Ile Arg Leu Thr Gln Ala Lys Gly His Glu Ala Thr Asn Gly Val

2735

2740

2745

caa atc gcc aac gac gcc cca ttc caa ctc att tcc gta gaa gat cca 8304

Gln Ile Ala Asn Asp Ala Pro Phe Gln Leu Ile Ser Val Glu Asp Pro

2750

2755

2760

gag ata ttc gtc caa cgt gaa atc gcc cct caa cta caa tgc tca ccc 8352

Glu Ile Phe Val Gln Arg Glu Ile Ala Pro Gln Leu Gln Cys Ser Pro

2765

2770

2775

2780

gag aca atc cta gac gtc tac ccc gcc acg caa atg caa agg gtc ttc 8400

Glu Thr Ile Leu Asp Val Tyr Pro Ala Thr Gln Met Gln Arg Val Phe

2785

2790

2795

ctc ctc aac cca gta aca gga aag ccg cgc tca cca acg cca ttt cac 8448

Leu Leu Asn Pro Val Thr Gly Lys Pro Arg Ser Pro Thr Pro Phe His

2800

2805

2810

ata gac ttc ccg ccg gac gca gac tgc gcc agc ctg atg cgg gca tgt 8496

Ile Asp Phe Pro Pro Asp Ala Asp Cys Ala Ser Leu Met Arg Ala Cys

2815

2820

2825

gca tct cta gcg aag cat ttc gat atc ttc agg acg gtg ttc ctc gaa 8544

Ala Ser Leu Ala Lys His Phe Asp Ile Phe Arg Thr Val Phe Leu Glu

2830

2835

2840

gcc aga ggc gaa ctc tac caa gta gtt cta aaa cac gtc gac gtg ccc 8592

Ala Arg Gly Glu Leu Tyr Gln Val Val Leu Lys His Val Asp Val Pro

2845

2850

2855

2860

atc gag atg ctc cag acc gaa gaa aac atc aac agc gcg acc cgg tcg 8640

Ile Glu Met Leu Gln Thr Glu Glu Asn Ile Asn Ser Ala Thr Arg Ser

2865

2870

2875

ttc ctg gac gta gac gca gaa aaa ccc atc cgg cta ggc cag cca ctg 8688

Phe Leu Asp Val Asp Ala Glu Lys Pro Ile Arg Leu Gly Gln Pro Leu

2880

2885

2890

atc cgc atc gcg ata cta gag aag ccc ggg tcc acg ctg cgc gtc atc 8736

Ile Arg Ile Ala Ile Leu Glu Lys Pro Gly Ser Thr Leu Arg Val Ile

2895

2900

2905

cta cga tta tcc cac gcc tta tac gac ggc ctg agc cta gag cac atc 8784

Leu Arg Leu Ser His Ala Leu Tyr Asp Gly Leu Ser Leu Glu His Ile

2910

2915

2920

ctg cac tct ctg cac atc ctc ttt ttc ggc ggc agt ctt ccc ccg ccg 8832

Leu His Ser Leu His Ile Leu Phe Phe Gly Gly Ser Leu Pro Pro Pro

2925

2930

2935

2940

ccc aag ttc gcc ggg tac atg caa cac gtc gcg agc agt cgc aga gaa 8880

Pro Lys Phe Ala Gly Tyr Met Gln His Val Ala Ser Ser Arg Arg Glu

2945

2950

2955

ggc tac gat ttc tgg cgt tcc gtt ctc cga gat tcg tct atg aca gtc 8928

Gly Tyr Asp Phe Trp Arg Ser Val Leu Arg Asp Ser Ser Met Thr Val

2960

2965

2970

atc aaa ggc aac aat aat aca act cca cca cct cct cct caa caa caa 8976

Ile Lys Gly Asn Asn Asn Thr Thr Pro Pro Pro Pro Pro Gln Gln Gln

2975

2980

2985

tcc acc ccc tcc gga gcc cac cac gcc tcc aaa gta gtc act atc cca 9024

Ser Thr Pro Ser Gly Ala His His Ala Ser Lys Val Val Thr Ile Pro

2990

2995

3000

acc caa gcc aac aca gac agc cgg atc acg cgc gcc acg atc ttc act 9072

Thr Gln Ala Asn Thr Asp Ser Arg Ile Thr Arg Ala Thr Ile Phe Thr

3005

3010

3015

3020

acc gct tgc gca cta atg ctc gcg aaa gaa gac aac tcc agc gac gtc 9120

Thr Ala Cys Ala Leu Met Leu Ala Lys Glu Asp Asn Ser Ser Asp Val

3025

3030

3035

gtc ttc ggg cgt acg gta tcg ggg cgt caa ggc ctg ccc cta gcc cac 9168

Val Phe Gly Arg Thr Val Ser Gly Arg Gln Gly Leu Pro Leu Ala His

3040

3045

3050

caa aac gtg atc gga cca tgt ctc aac caa gtg ccc gtg cgc gcg cgc 9216

Gln Asn Val Ile Gly Pro Cys Leu Asn Gln Val Pro Val Arg Ala Arg

3055

3060

3065

ggt tta aac cga gga acc act cac cac cga gaa ctt ctc cgc gag atg 9264

Gly Leu Asn Arg Gly Thr Thr His His Arg Glu Leu Leu Arg Glu Met

3070

3075

3080

caa gag caa tat ctc aac agt ctc gct ttc gaa act ctc ggg tac gac 9312

Gln Glu Gln Tyr Leu Asn Ser Leu Ala Phe Glu Thr Leu Gly Tyr Asp

3085

3090

3095

3100

gag atc aag gcg cac tgc aca gac tgg ccg gac gtg cca gcg acc gcg 9360

Glu Ile Lys Ala His Cys Thr Asp Trp Pro Asp Val Pro Ala Thr Ala

3105

3110

3115

agc ttc ggg tgc tgc atc gtg tac cag aac ttc gat tcg cac ccg gac 9408

Ser Phe Gly Cys Cys Ile Val Tyr Gln Asn Phe Asp Ser His Pro Asp

3120

3125

3130

agc cga gtc gaa gag cag cgg ctg cag atc ggg gtc ttg tgc cgg aac 9456

Ser Arg Val Glu Glu Gln Arg Leu Gln Ile Gly Val Leu Ser Arg Asn

3135

3140

3145

tac gag gct att aat gag ggg ctc gtg cat gat ctt gtt att gct ggg 9504

Tyr Glu Ala Ile Asn Glu Gly Leu Val His Asp Leu Val Ile Ala Gly

3150

3155

3160

gag tgc gag cct gat ggg gat gat ttg agg gtt act gtt gtg gcg aat 9552

Glu Ser Glu Pro Asp Gly Asp Asp Leu Arg Val Thr Val Val Ala Asn

3165

3170

3175

3180

cgg agg ttg tgc gat gag gaa agg ttg aag agg atg ctg gag gag ctg 9600

Arg Arg Leu Cys Asp Glu Glu Arg Leu Lys Arg Met Leu Glu Glu Leu

3185

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3195

tgt ggg aat att cgg gct ttg gcg ttg gtt tag

9633

Cys Gly Asn Ile Arg Ala Leu Ala Leu Val

3200

3205

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<211> 3210

<212> PRT

<213> Mycelia sterilia

<400> 2

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1

5

10

15

Leu Ser Leu Ser Cys Pro Leu Leu Pro His Asp Asp Glu Lys His Ser

20

25

30

Asp Asn Leu Tyr Glu Gln Ala Thr Arg His Phe Gly Leu Ser Arg Asp

35

40

45

Lys Ile Glu Asn Val Leu Pro Cys Thr Ser Phe Gln Cys Asp Val Ile

50

55

60

Asp Cys Ala Val Asp Asp Arg Arg His Ala Ile Gly His Val Val Tyr

65

70

75

80

Asp Ile Pro Asn Thr Val Asp Ile Gln Arg Leu Ala Ala Ala Trp Lys

85

90

95

Glu Val Val Arg Gln Thr Pro Ile Leu Arg Thr Gly Ile Phe Thr Ser

100

105

110

Glu Thr Gly Asp Ser Phe Gln Ile Val Leu Lys Glu Gly Cys Leu Pro

115

120

125

Trp Met Tyr Ala Thr Cys Leu Gly Met Lys Gly Ala Val Ile Gln Asp

130

135

140

Glu Ala Val Ala Ala Met Thr Gly Pro Arg Cys Asn Arg Tyr Val Val

145

150

155

160

Leu Glu Asp Pro Ser Thr Lys Gln Arg Leu Leu Ile Trp Thr Phe Ser

165

170

175

His Ala Leu Val Asp Tyr Thr Val Gln Glu Arg Ile Leu Gln Arg Val

180

185

190

Leu Thr Val Tyr Asp Gly Arg Asp Val Glu Cys Pro Arg Ile Lys Asp

195

200

205

Thr Glu His Val Ser Arg Phe Trp Gln Gln His Phe Glu Gly Leu Asp

210

215

220

Ala Ser Val Phe Pro Leu Leu Pro Ser His Leu Thr Val Cys Asn Pro

225

230

235

240

Asn Ala Arg Ala Glu His His Ile Ser Tyr Thr Gly Pro Val Gln Arg

245

250

255

Lys Trp Ser His Thr Ser Ile Cys Arg Ala Ala Leu Ala Val Leu Leu

260

265

270

Ser Arg Phe Thr His Ser Ser Glu Ala Leu Phe Gly Val Val Thr Glu

275

280

285

Gln Ser His Asn Ser Glu Asp Gln Arg Arg Ser Ile Asp Gly Pro Ala

290

295

300

Arg Thr Val Val Pro Ile Arg Val Leu Cys Ala Pro Asp Gln Tyr Val

305

310

315

320

Ser Asp Val Ile Gly Ala Ile Thr Ala His Glu His Ala Met Arg Gly

325

330

335

Phe Glu His Ala Gly Leu Arg Asn Ile Arg Arg Thr Gly Asp Asp Gly

340

345

350

Ser Ala Ala Cys Gly Phe Gln Thr Val Leu Leu Val Thr Asp Gly Asp

355

360

365

Ala Pro Lys Thr Pro Gly Ser Val Leu His Arg Ser Val Glu Glu Ser

370

375

380

Asp Arg Phe Met Pro Cys Ala Asn Arg Ala Leu Leu Leu Asp Cys Gln

385

390

395

400

Met Ala Gly Asn Ser Ala Ser Leu Val Ala Arg Tyr Asp His Asn Val

405

410

415

Ile Asp Pro Arg Gln Met Ser Arg Phe Leu Arg Gln Leu Gly Tyr Leu

420

425

430

Ile Gln Gln Phe His His His Val Asp Leu Pro Leu Val Lys Glu Leu

435

440

445

Asp Val Val Thr Ala Glu Asp Cys Ala Glu Ile Glu Lys Trp Asn Ser

450

455

460

Glu Arg Leu Thr Met Gln Asp Ala Leu Ile His Asp Thr Ile Ser Lys

465

470

475

480

Trp Ala Ala Gly Asp Pro Asn Lys Ala Ala Val Phe Ala Trp Asp Gly

485

490

495

Glu Trp Thr Tyr Ala Glu Leu Asp Asn Ile Ser Ser Arg Leu Ala Val

500

505

510

Tyr Ile Gln Ser Leu Asp Leu Arg Pro Gly Gln Ala Ile Leu Pro Leu

515

520

525

Cys Phe Glu Lys Ser Lys Trp Val Val Ala Thr Ile Leu Ala Val Leu

530

535

540

Lys Val Gly Arg Ala Phe Thr Leu Ile Asp Pro Cys Asp Pro Ser Ala

545

550

555

560

Arg Met Ala Gln Val Cys Gln Gln Thr Ser Ala Thr Val Ala Leu Thr

565

570

575

Ser Lys Leu His Asn Thr Thr Leu Arg Ser Val Val Ser Arg Cys Ile

580

585

590

Val Val Asp Asp Asp Leu Leu Arg Ser Leu Pro His Ala Asp Gly Arg

595

600

605

Leu Lys Ala Thr Val Lys Pro Gln Asp Leu Ala Tyr Val Ile Phe Thr

610

615

620

Ser Gly Ser Thr Gly Glu Pro Lys Gly Ile Met Ile Glu His Arg Gly

625

630

635

640

Phe Val Ser Cys Ala Met Lys Phe Gly Pro Ala Leu Gly Met Asp Glu

645

650

655

His Thr Arg Ala Leu Gln Phe Ala Ser Tyr Ala Phe Gly Ala Cys Leu

660

665

670

Val Glu Val Val Thr Ala Leu Met His Gly Gly Cys Val Cys Ile Pro

675

680

685

Ser Asp Asp Asp Arg Leu Asn Asn Val Pro Glu Phe Ile Lys Arg Ala

690

695

700

Gln Val Asn Trp Val Ile Leu Thr Pro Ser Tyr Ile Gly Thr Phe Gln

705

710

715

720

Pro Glu Asp Val Pro Gly Leu Gln Thr Leu Val Leu Val Gly Glu Pro

725

730

735

Ile Ser Ala Ser Ile Arg Asp Thr Trp Ala Ser Gln Val Arg Leu Leu

740

745

750

Asn Ala Tyr Gly Gln Ser Glu Ser Ser Thr Met Cys Ser Val Thr Glu

755

760

765

Val Ser Pro Leu Ser Leu Glu Pro Asn Asn Ile Gly Arg Ala Val Gly

770

775

780

Ala Arg Ser Trp Ile Ile Asp Pro Asp Glu Pro Asp Arg Leu Ala Pro

785

790

795

800

Ile Gly Cys Ile Gly Glu Leu Val Ile Glu Ser Pro Gly Ile Ala Arg

805

810

815

Asp Tyr Ile Ile Ala Pro Pro Pro Asp Lys Ser Pro Phe Leu Leu Ala

820

825

830

Pro Pro Ala Trp Tyr Pro Ala Gly Lys Leu Ser Asn Ala Phe Lys Phe

835

840

845

Tyr Lys Thr Gly Asp Leu Val Arg Tyr Gly Pro Asp Gly Thr Ile Val

850

855

860

Cys Leu Gly Arg Lys Asp Ser Gln Val Lys Ile Arg Gly Gln Arg Val

865

870

875

880

Glu Ile Ser Ala Val Glu Ala Ser Leu Arg Arg Gln Leu Pro Ser Asp

885

890

895

Ile Met Pro Val Ala Glu Ala Ile Lys Arg Ser Asp Ser Ser Gly Ser

900

905

910

Thr Val Leu Thr Ala Phe Leu Ile Gly Ser Ser Lys Ser Gly Asp Gly

915

920

925

Asn Gly His Ala Leu Ser Ala Ala Asp Ala Val Ile Leu Asp His Gly

930

935

940

Ala Thr Asn Glu Ile Asn Ala Lys Leu Gln Gln Ile Leu Pro Gln His

945

950

955

960

Ser Val Pro Ser Tyr Tyr Ile His Met Glu Asn Leu Pro Arg Thr Ala

965

970

975

Thr Gly Lys Ala Asp Arg Lys Met Leu Arg Ser Ile Ala Ser Lys Leu

980

985

990

Leu Gly Glu Leu Ser Gln Asn Val Thr Ser Gln Pro Ile Glu Lys His

995

1000

1005

Asp Ala Pro Ala Thr Gly Ile Glu Val Lys Leu Lys Glu Leu Trp Phe

1010

1015

1020

Leu Ser Leu Asn Leu Asn Pro Asn Ser Gln Asp Val Gly Ala Ser Phe

025

1030

1035

1040

Phe Asp Leu Gly Gly Asn Ser Ile Ile Ala Ile Lys Met Val Asn Met

1045

1050

1055

Ala Arg Ser Ala Gly Ile Ala Leu Lys Val Ser Asp Ile Phe Gln Asn

1060

1065

1070

Pro Thr Leu Ala Gly Leu Val Asp Val Ile Gly Arg Asp Pro Ala Pro

1075

1080

1085

Tyr Asn Leu Ile Pro Thr Thr Ala Tyr Ser Gly Pro Val Glu Gln Ser

1090

1095

1100

Phe Ala Gln Gly Arg Leu Trp Phe Leu Asp Gln Ile Glu Leu Asp Ala

105

1110

1115

1120

Leu Trp Tyr Leu Leu Pro Tyr Ala Val Arg Met Arg Gly Pro Leu His

1125

1130

1135

Ile Asp Ala Leu Thr Ile Ala Leu Leu Ala Ile Gln Gln Arg His Glu

1140

1145

1150

Thr Leu Arg Thr Thr Phe Glu Glu Gln Asp Gly Val Gly Val Gln Val

1155

1160

1165

Val His Ala Ser Pro Ile Ser Asp Leu Arg Ile Ile Asp Val Ser Gly

1170

1175

1180

Asp Arg Asn Ser Asp Tyr Leu Gln Leu Leu His Gln Glu Gln Thr Thr

185

1190

1195

1200

Pro Phe Ile Leu Ala Cys Gln Ala Gly Trp Arg Val Ser Leu Ile Arg

1205

1210

1215

Leu Gly Glu Asp Asp His Ile Leu Ser Ile Val Met His His Ile Ile

1220

1225

1230

Ser Asp Gly Trp Ser Ile Asp Ile Leu Arg Arg Glu Leu Ser Asn Phe

1235

1240

1245

Tyr Ser Ala Ala Leu Arg Gly Ser Asp Pro Leu Ser Val Val Ser Pro

1250

1255

1260

Leu Pro Leu His Tyr Arg Asp Phe Ser Val Trp Gln Lys Gln Val Glu

265

1270

1275

1280

Gln Glu Thr Glu His Glu Arg Gln Leu Glu Tyr Trp Val Lys Gln Leu

1285

1290

1295

Ala Asp Ser Ser Ala Ala Glu Phe Leu Thr Asp Phe Pro Arg Pro Asn

1300

1305

1310

Ile Leu Ser Gly Glu Ala Gly Ser Val Pro Val Thr Ile Glu Gly Glu

1315

1320

1325

Leu Tyr Glu Arg Leu Gln Glu Phe Cys Lys Val Glu Gln Met Thr Pro

1330

1335

1340

Phe Ala Val Leu Leu Gly Ala Phe Arg Ala Thr His Tyr Arg Leu Thr

345

1350

1355

1360

Gly Ala Glu Asp Ser Ile Ile Gly Thr Pro Ile Ala Asn Arg Asn Arg

1365

1370

1375

Gln Glu Leu Glu Asn Met Ile Gly Phe Phe Val Asn Thr Gln Cys Met

1380

1385

1390

Arg Ile Thr Val Asp Gly Asp Asp Thr Phe Glu Ser Leu Val Arg Gln

1395

1400

1405

Val Arg Thr Thr Ala Thr Ala Ala Phe Glu His Gln Asp Val Pro Phe

1410

1415

1420

Glu Arg Val Val Thr Ala Leu Leu Pro Arg Ser Arg Asp Leu Ser Arg

425

1430

1435

1440

Asn Pro Leu Ala Gln Leu Thr Phe Ala Leu His Ser Gln Gln Asp Leu

1445

1450

1455

Gly Lys Phe Glu Leu Glu Gly Leu Val Ala Glu Pro Val Ser Asn Lys

1460

1465

1470

Val Tyr Thr Arg Phe Asp Val Glu Phe His Leu Phe Gln Glu Ala Gly

1475

1480

1485

Arg Leu Ser Gly Asn Val Ala Phe Ala Ala Asp Leu Phe Lys Pro Glu

1490

1495

1500

Thr Ile Ser Asn Val Val Ala Ile Phe Phe Gln Ile Leu Arg Gln Gly

505

1510

1515

1520

Ile Arg Gln Pro Arg Thr Pro Ile Ala Val Leu Pro Leu Thr Asp Gly

1525

1530

1535

Leu Ala Asp Leu Arg Ala Met Gly Leu Leu Glu Ile Glu Lys Ala Glu

1540

1545

1550

Tyr Pro Arg Glu Ser Ser Val Val Asp Val Phe Arg Lys Gln Val Ala

1555

1560

1565

Ala His Pro His Ala Phe Ala Val Val Asp Ser Ala Ser Arg Leu Thr

1570

1575

1580

Tyr Ala Asp Leu Asp Arg Gln Ser Asp Gln Leu Ala Thr Trp Leu Gly

585

1590

1595

1600

Arg Arg Asn Met Thr Ala Glu Thr Leu Val Gly Val Leu Ala Pro Arg

1605

1610

1615

Ser Cys Gln Thr Val Val Ala Ile Leu Gly Ile Leu Lys Ala Asn Leu

1620

1625

1630

Ala Tyr Leu Pro Leu Asp Val Asn Cys Pro Thr Ala Arg Leu Gln Thr

1635

1640

1645

Ile Leu Ser Thr Leu Asn Arg His Lys Leu Val Leu Leu Gly Ser Asn

1650

1655

1660

Ala Thr Thr Pro Asp Val Gln Ile Pro Asp Val Glu Leu Val Arg Ile

665

1670

1675

1680

Ser Asp Ile Leu Asp Arg Pro Ile Asn Gly Gln Ala Lys Leu Asn Gly

1685

1690

1695

His Thr Lys Ser Asn Gly Tyr Ser Lys Pro Asn Gly Tyr Thr His Leu

1700

1705

1710

Lys Gly Tyr Ser Asn Leu Asn Gly Tyr Ser Lys Gln Asn Gly Tyr Ala

1715

1720

1725

Gln Leu Asn Gly His Arg Glu Arg Asn Asn Tyr Leu Asp Leu Asn Gly

1730

1735

1740

His Ser Leu Leu Asn Gly Asn Ser Asp Ile Thr Thr Ser Gly Pro Ser

745

1750

1755

1760

Ala Thr Ser Leu Ala Tyr Val Ile Phe Thr Ser Gly Ser Thr Gly Lys

1765

1770

1775

Pro Lys Gly Val Met Val Glu His Arg Ser Ile Ile Arg Leu Ala Lys

1780

1785

1790

Lys Asn Arg Ile Ile Ser Arg Phe Pro Ser Val Ala Lys Val Ala His

1795

1800

1805

Leu Ser Asn Ile Ala Phe Asp Ala Ala Thr Trp Glu Met Phe Ala Ala

1810

1815

1820

Leu Leu Asn Gly Gly Thr Leu Val Cys Ile Asp Tyr Met Thr Thr Leu

825

1830

1835

1840

Asp Ser Lys Thr Leu Glu Ala Ala Phe Ala Arg Glu Gln Ile Asn Ala

1845

1850

1855

Ala Leu Leu Thr Pro Ala Leu Leu Lys Gln Cys Leu Ala Asn Ile Pro

1860

1865

1870

Thr Thr Leu Gly Arg Leu Ser Ala Leu Val Ile Gly Gly Asp Arg Leu

1875

1880

1885

Asp Gly Gln Asp Ala Ile Ala Ala His Ala Leu Val Gly Ala Gly Val

1890

1895

1900

Tyr Asn Ala Tyr Gly Pro Thr Glu Asn Gly Val Ile Ser Thr Ile Tyr

905

1910

1915

1920

Asn Ile Thr Lys Asn Asp Ser Phe Ile Asn Gly Val Pro Ile Gly Cys

1925

1930

1935

Ala Ile Ser Asn Ser Gly Ala Tyr Ile Thr Asp Pro Asp Gln Gln Leu

1940

1945

1950

Val Pro Pro Gly Val Met Gly Glu Leu Val Val Thr Gly Asp Gly Leu

1955

1960

1965

Ala Arg Gly Tyr Thr Asp Pro Ala Leu Asp Ala Gly Arg Phe Val Gln

1970

1975

1980

Ile Met Ile Asn Asp Lys Ala Val Arg Ala Tyr Arg Thr Gly Asp Arg

985

1990

1995

2000

Ala Arg Tyr Arg Val Gly Asp Gly Gln Ile Glu Phe Phe Gly Arg Met

2005

2010

2015

Asp Gln Gln Val Lys Ile Arg Gly His Arg Ile Glu Pro Ala Glu Val

2020

2025

2030

Glu Arg Ala Ile Leu Asp Gln Asp Ser Ala Arg Asp Ala Val Val Val

2035

2040

2045

Ile Arg His Gln Glu Gly Glu Glu Pro Glu Met Val Gly Phe Val Ala

2050

2055

2060

Thr His Gly Asp His Ser Ala Glu Gln Glu Glu Ala Asp Asp Gln Val

065

2070

2075

2080

Glu Gly Trp Lys Asp Phe Phe Glu Ser Asn Thr Tyr Ala Asp Met Asp

2085

2090

2095

Thr Ile Gly Gln Ser Ala Ile Gly Asn Asp Phe Thr Gly Trp Thr Ser

2100

2105

2110

Met Tyr Asp Gly Ser Glu Ile Asn Lys Ala Glu Met Gln Glu Trp Leu

2115

2120

2125

Asp Asp Thr Met Arg Thr Leu Leu Asp Gly Gln Ala Pro Gly His Val

2130

2135

2140

Leu Glu Ile Gly Thr Gly Ser Gly Met Val Leu Phe Asn Leu Gly Ala

145

2150

2155

2160

Gly Leu Gln Ser Tyr Val Gly Leu Glu Pro Ser Arg Ser Ala Ala Thr

2165

2170

2175

Phe Val Thr Lys Ala Ile Asn Ser Thr Pro Ala Leu Ala Gly Lys Ala

2180

2185

2190

Glu Val His Val Gly Thr Ala Thr Asp Ile Asn Arg Leu Arg Gly Leu

2195

2200

2205

Arg Pro Asp Leu Val Val Leu Asn Ser Val Val Gln Tyr Phe Pro Thr

2210

2215

2220

Pro Glu Tyr Leu Leu Glu Val Val Glu Ser Leu Val Arg Ile Pro Gly

2225

2230

2235

2240

Val Lys Arg Val Val Phe Gly Asp Ile Arg Ser His Ala Thr Asn Arg

2245

2250

2255

His Phe Leu Ala Ala Arg Ala Leu His Ser Leu Gly Ser Lys Ala Thr

2260

2265

2270

Lys Asp Ala Ile Arg Gln Lys Met Thr Glu Met Glu Glu Arg Glu Glu

2275

2280

2285

Glu Leu Leu Val Asp Pro Ala Phe Phe Thr Ala Leu Leu Gln Gly Gln

2290

2295

2300

Leu Ala Asp Arg Ile Lys His Val Glu Ile Leu Pro Lys Asn Met Arg

305

2310

2315

2320

Ala Thr Asn Glu Leu Ser Ala Tyr Arg Tyr Thr Ala Val Ile His Val

2325

2330

2335

Arg Gly Pro Glu Glu Gln Ser Arg Pro Val Tyr Pro Ile Gln Val Asn

2340

2345

2350

Asp Trp Ile Asp Phe Gln Ala Ser Arg Ile Asp Arg Arg Ala Leu Leu

2355

2360

2365

Arg Leu Leu Gln Arg Ser Ala Asp Ala Ala Thr Val Ala Val Ser Asn

2370

2375

2380

Ile Pro Tyr Ser Lys Thr Ile Val Glu Arg His Val Val Glu Ser Leu

385

2390

2395

2400

Asp Asn Asn Asn Arg Glu Asn Thr His Arg Ala Pro Asp Gly Ala Ala

2405

2410

2415

Trp Ile Ser Ala Val Arg Ser Lys Ala Glu Arg Cys Thr Ser Leu Ser

2420

2425

2430

Val Thr Asp Leu Val Gln Leu Gly Glu Glu Ala Gly Phe Arg Val Glu

2435

2440

2445

Val Ser Ala Ala Arg Gln Trp Ser Gln Ser Gly Ala Leu Asp Ala Val

2450

2455

2460

Phe His Arg Tyr Asn Leu Pro Thr Gln Ser Asn Ser Arg Val Leu Ile

465

2470

2475

2480

Gln Phe Pro Thr Glu Asp Gly Gln Thr Arg Arg Ser Ala Thr Leu Thr

2485

2490

2495

Asn Arg Pro Leu Gln Arg Leu Gln Ser Arg Arg Phe Ala Ser Gln Ile

2500

2505

2510

Arg Glu Gln Leu Lys Ala Val Leu Pro Ser Tyr Met Ile Pro Ser Arg

2515

2520

2525

Ile Val Val Ile Asp Gln Met Pro Leu Asn Ala Asn Gly Lys Val Asp

2530

2535

2540

Arg Lys Glu Leu Thr Arg Arg Ala Gln Ile Ala Pro Lys Ser Gln Ala

545

2550

2555

2560

Ala Pro Ala Lys Pro Val Lys Gln Val Asp Pro Phe Val Asn Leu Glu

2565

2570

2575

Ala Ile Leu Cys Glu Glu Phe Ala Glu Val Leu Gly Met Glu Val Gly

2580

2585

2590

Val Asn Asp His Phe Phe Gln Leu Gly Gly His Ser Leu Leu Ala Thr

2595

2600

2605

Lys Leu Val Ala Arg Leu Ser Arg Arg Leu Asn Gly Arg Val Ser Val

2610

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2620

Arg Asp Val Phe Asp Gln Pro Val Ile Ser Asp Leu Ala Val Thr Leu

2625

2630

2635

2640

Arg Gln Gly Leu Thr Leu Glu Asn Ala Ile Pro Ala Thr Pro Asp Ser

2645

2650

2655

Gly Tyr Trp Glu Gln Thr Met Ser Ala Pro Thr Thr Pro Ser Asp Asp

2660

2665

2670

Met Glu Ala Val Leu Cys Lys Glu Phe Ala Asp Val Leu Gly Val Glu

2675

2680

2685

Val Ser Ala Thr Asp Ser Phe Phe Asp Leu Gly Gly His Ser Leu Met

2690

2695

2700

Ala Thr Lys Leu Ala Ala Arg Ile Ser Arg Arg Leu Asp Val Pro Val

2705

2710

2715

2720

Ser Ile Lys Asp Ile Phe Asp His Ser Val Pro Leu Asn Leu Ala Arg

2725

2730

2735

Lys Ile Arg Leu Thr Gln Ala Lys Gly His Glu Ala Thr Asn Gly Val

2740

2745

2750

Gln Ile Ala Asn Asp Ala Pro Phe Gln Leu Ile Ser Val Glu Asp Pro

2755

2760

2765

Glu Ile Phe Val Gln Arg Glu Ile Ala Pro Gln Leu Gln Cys Ser Pro

2770

2775

2780

Glu Thr Ile Leu Asp Val Tyr Pro Ala Thr Gln Met Gln Arg Val Phe

785

2790

2795

2800

Leu Leu Asn Pro Val Thr Gly Lys Pro Arg Ser Pro Thr Pro Phe His

2805

2810

2815

Ile Asp Phe Pro Pro Asp Ala Asp Cys Ala Ser Leu Met Arg Ala Cys

2820

2825

2830

Ala Ser Leu Ala Lys His Phe Asp Ile Phe Arg Thr Val Phe Leu Glu

2835

2840

2845

Ala Arg Gly Glu Leu Tyr Gln Val Val Leu Lys His Val Asp Val Pro

2850

2855

2860

Ile Glu Met Leu Gln Thr Glu Glu Asn Ile Asn Ser Ala Thr Arg Ser

865

2870

2875

2880

Phe Leu Asp Val Asp Ala Glu Lys Pro Ile Arg Leu Gly Gln Pro Leu

2885

2890

2895

Ile Arg Ile Ala Ile Leu Glu Lys Pro Gly Ser Thr Leu Arg Val Ile

2900

2905

2910

Leu Arg Leu Ser His Ala Leu Tyr Asp Gly Leu Ser Leu Glu His Ile

2915

2920

2925

Leu His Ser Leu His Ile Leu Phe Phe Gly Gly Ser Leu Pro Pro Pro

2930

2935

2940

Pro Lys Phe Ala Gly Tyr Met Gln His Val Ala Ser Ser Arg Arg Glu

945

2950

2955

2960

Gly Tyr Asp Phe Trp Arg Ser Val Leu Arg Asp Ser Ser Met Thr Val

2965

2970

2975

Ile Lys Gly Asn Asn Asn Thr Thr Pro Pro Pro Pro Pro Gln Gln Gln

2980

2985

2990

Ser Thr Pro Ser Gly Ala His His Ala Ser Lys Val Val Thr Ile Pro

2995

3000

3005

Thr Gln Ala Asn Thr Asp Ser Arg Ile Thr Arg Ala Thr Ile Phe Thr

3010

3015

3020

Thr Ala Cys Ala Leu Met Leu Ala Lys Glu Asp Asn Ser Ser Asp Val

025

3030

3035

3040

Val Phe Gly Arg Thr Val Ser Gly Arg Gln Gly Leu Pro Leu Ala His

3045

3050

3055

Gln Asn Val Ile Gly Pro Cys Leu Asn Gln Val Pro Val Arg Ala Arg

3060

3065

3070

Gly Leu Asn Arg Gly Thr Thr His His Arg Glu Leu Leu Arg Glu Met

3075

3080

3085

Gln Glu Gln Tyr Leu Asn Ser Leu Ala Phe Glu Thr Leu Gly Tyr Asp

3090

3095

3100

Glu Ile Lys Ala His Cys Thr Asp Trp Pro Asp Val Pro Ala Thr Ala

105

3110

3115

3120

Ser Phe Gly Cys Cys Ile Val Tyr Gln Asn Phe Asp Ser His Pro Asp

3125

3130

3135

Ser Arg Val Glu Glu Gln Arg Leu Gln Ile Gly Val Leu Ser Arg Asn

3140

3145

3150

Tyr Glu Ala Ile Asn Glu Gly Leu Val His Asp Leu Val Ile Ala Gly

3155

3160

3165

Glu Ser Glu Pro Asp Gly Asp Asp Leu Arg Val Thr Val Val Ala Asn

3170

3175

3180

Arg Arg Leu Cys Asp Glu Glu Arg Leu Lys Arg Met Leu Glu Glu Leu

185

3190

3195

3200

Cys Gly Asn Ile Arg Ala Leu Ala Leu Val

3205

3210

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<210> 4

<211> 20

<212> DNA

<213> Artificial Sequence

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<211> 30

<212> DNA

<213> Artificial Sequence

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<211> 30

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<213> Artificial Sequence

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<212> DNA

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<223> Description of Artificial Sequence:synthetic DNA

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20

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42

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<212> DNA

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<210> 12

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<223> Description of Artificial Sequence:synthetic DNA

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agcatcggat cctaacaatg tcaaacatgg caccactccc ta

42

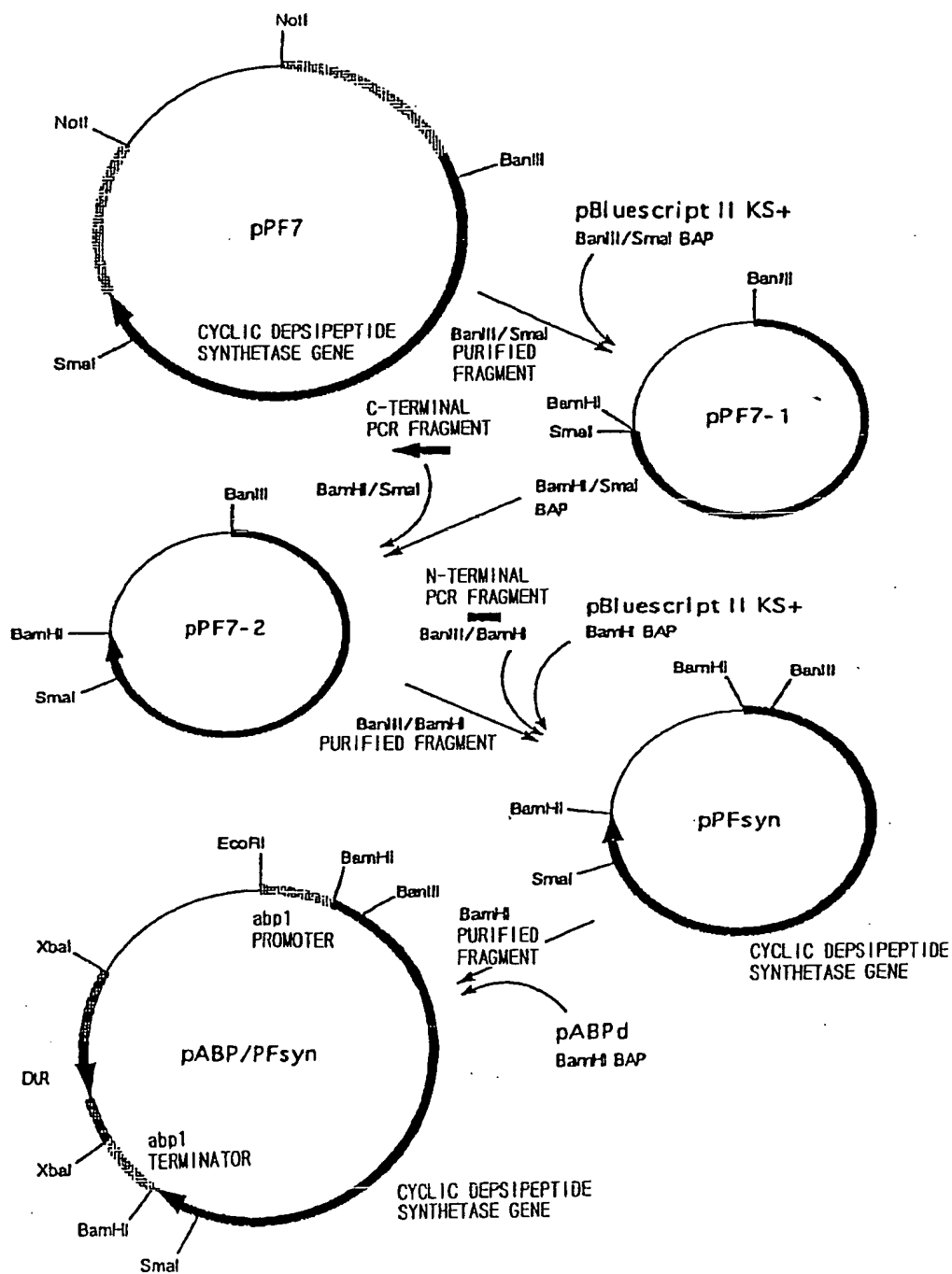
[Brief description of the drawings]

[Figure 1]

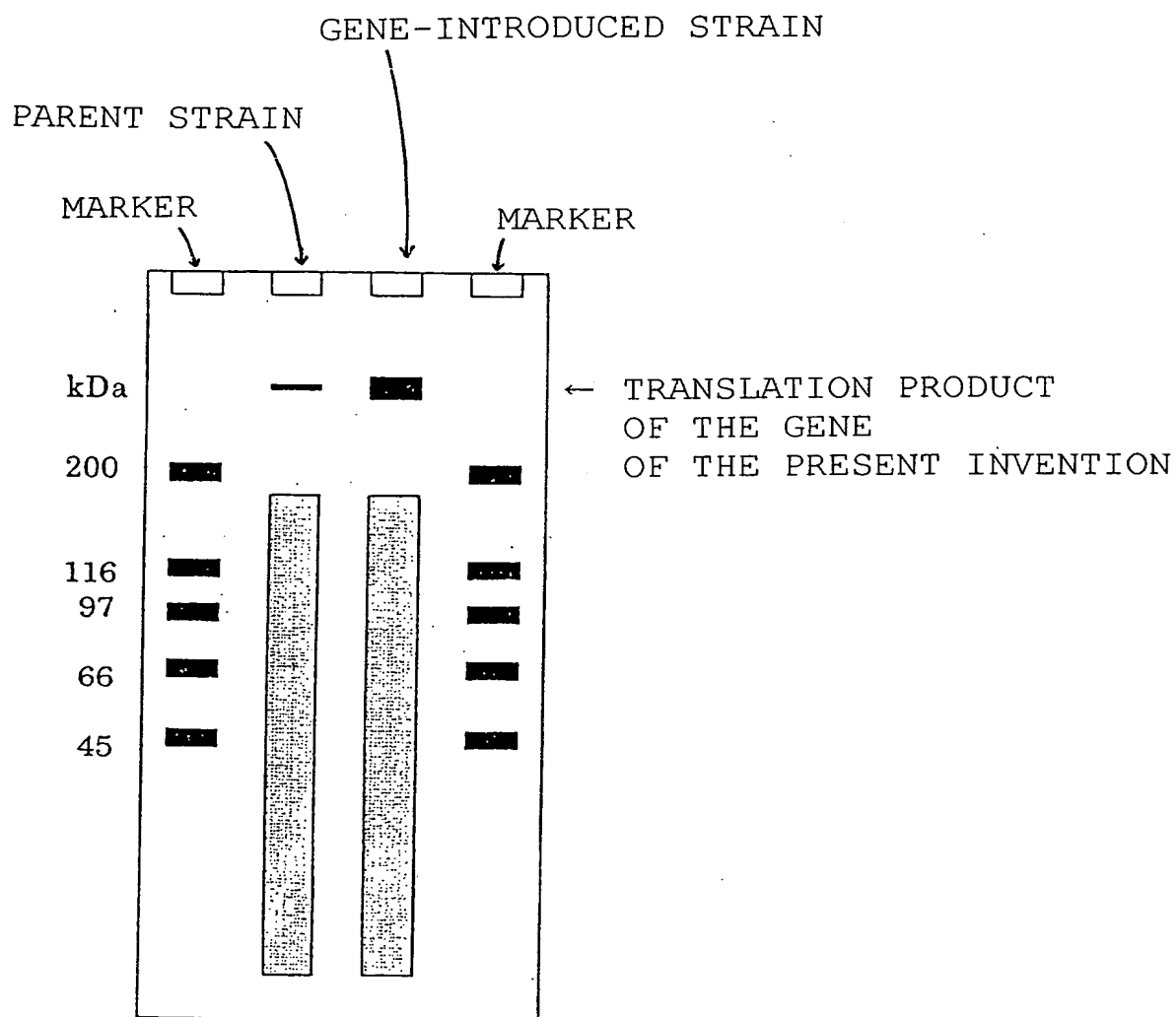
Figure 1 shows a construction procedure of plasmid pABP/PFsyn.

[Figure 2]

Figure 2 schematically shows the SDS-PAGE result of the expression, in a gene-introduced strain, of the cyclic depsipeptide synthetase which is the translation product of the gene of the present invention.



[Figure 2]



[TTITLE OF DOCUMENT] ABSTRACT

[ABSTRACT]

[OBJECT OF INVENTION]

To provide a method for producing a cyclic depsipeptide having anthelmintic activity, a cyclic depsipeptide synthetase gene capable of improving productivity of the substance PF1022 and a protein coding therefor, a recombinant vector comprising the gene, and a substance PF1022-producing microorganism and substance PF1022 into which the recombinant vector is introduced.

[MEANS FOR ATTAINING THE OBJECT]

A gene encoding a cyclic depsipeptide synthetase enhancing the biosynthesis of the substance PF1022 is isolated from a substance PF1022-producing microorganism. A recombinant vector is prepared by the substitution of the promoter and terminator of the gene with those of a foreign gene utilizable for expression augmentation, and then the recombinant vector is introduced into the substance PF 1022-producing microorganism, whereby the improvement in productivity of the substance PF 1022 was attained.

[SELECTED FIGURE]

None